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# Magnesium Ameliorates Aluminum Rhizotoxicity in Soybean by Increasing Citric Acid Production and Exudation by Roots

**Ivo R. Silva [1], Thomas J. Smyth [2, 5], Daniel W. Israel [3], Charles D. Raper [2] and Thomas W. Rufty [4]**

[1] *Department of Soil Science, Federal University of Viçosa, Viçosa, Minas Gerais, Brazil*
[2] *Department of Soil Science, North Carolina State University, Raleigh, North Carolina, U.S.A.*
[3] *USDA-ARS, Department of Soil Science, North Carolina State University, Raleigh, North Carolina, U.S.A.*
[4] *Department of Crop Science, North Carolina State University, Raleigh, North Carolina, U.S.A.*

**Superior effectiveness of Mg over Ca in alleviating Al rhizotoxicity cannot be accounted for by predicted changes in plasma membrane $Al^{3+}$ activity. The influence of Ca and Mg on the production and secretion of citrate and malate, and on Al accumulation by roots was investigated with soybean genotypes Young and PI 416937 which differ in Al tolerance. In the presence of a solution $Al^{3+}$ activity of 4.6 μM, citrate and malate concentrations of tap root tips of both genotypes increased with additions of either Ca up to 3 mM or Mg up to 50 μM. Citrate efflux rate from roots exposed to Al was only enhanced with Mg additions and exceeded malate efflux rates by as much as 50-fold. Maximum citrate release occurred within 12 h after adding Mg to solution treatments. Adding 50 μM Mg to 0.8 mM $CaSO_4$ solutions containing $Al^{3+}$ activities up to 4.6 μM increased citrate concentration of tap root tips by 3- to 5-fold and root exudation of citrate by 6- to 9-fold. Plants treated with either 50 μM Mg or 3 mM Ca had similar reductions in Al accumulation at tap root tips, which coincided with the respective ability of these ions to relieve Al rhizotoxicity. Amelioration of Al inhibition of soybean root elongation by low concentrations of Mg in solution involved Mg-stimulated production and efflux of citrate by roots.**

**Key words**: Aluminum tolerance — Calcium — Cation amelioration — Magnesium — Organic acids — Root exudates — Soybean.

Abbreviations: Al, aluminum; Ca, calcium; Mg, magnesium; Si, silicon.

## Introduction

Alleviation of Al toxicity by Ca and Mg has been reported for several plant species, either in soil (Gonzalez-Erico et al. 1979, Bruce et al. 1988, Tan et al. 1991) or solution culture (Kinraide et al. 1985, Kinraide and Parker 1987, Alva et al. 1986a, Alva et al. 1986b, Noble and Sumner 1988, Brady et al. 1993, Sanzonowicz et al. 1998a, Sanzonowicz et al. 1998b, Ferrufino et al. 2000). Nonetheless, the mechanism(s) through which Ca and Mg improve root growth in the presence of Al are not well understood. The most frequent explanation for the amelioration of Al toxicity by Ca and Mg is the reduction of Al activity through increased ionic strength of the solutions (Alva et al. 1986b, Noble and Sumner 1988, Wheeler and Edmeades 1995). Ameliorative effects of Ca and Mg also are observed, however, when $Al^{3+}$ activity in solution is kept constant (Alva et al. 1986a, Kinraide and Parker 1987, Brady et al. 1993, Lazof and Holland 1999, Silva et al. 2001b, Silva et al. 2001c), which suggests that other physiological processes are involved. Calcium and Mg alleviation of Al rhizotoxicity has been proposed to involve competition with Al for binding at sensitive sites in either the symplast or apoplast of root cells (Kinraide and Parker 1987), reduction in Al saturation in the root apoplast exchange sites (Grauer and Horst 1992), and decreased $Al^{3+}$ activity at the root cell plasma membrane surface (Kinraide 1994, Kinraide 1998).

Alleviation of Al rhizotoxicity in soybean with micromolar additions of Mg could not be accounted for by the cation's effect on root cell electrical potential and $Al^{3+}$ activities at the plasma-membrane surface, indicating that Mg, as opposed to Ca, involved non electrostatic mechanisms (Silva et al. 2001c). The mechanistic basis of this phenomenon is not presently known. Most of the work has been carried out with wheat, but there are indications that Mg and Ca amelioration of Al toxicity is dependent on species (Keltjens and Tan 1993) or even genotype (Hecht-Buchholz and Shuster 1987, Edmeades et al. 1991, Tan et al. 1992). Several experiments were conducted in the present investigation to compare the effects of Ca and Mg on the production and efflux of malate and citrate and Al accumulation by roots of soybean genotypes exposed to Al in hydroponics.

## Material and Methods

*Growth conditions*

Soybean seeds of cv Young and Plant Introduction 413967 (PI) were rolled in between sheets of germinating paper soaked with a 0.1 mM $CaSO_4$ solution and germinated in the dark at 25–26°C for 72 h in an incubator. After germination, seedlings with uniform root length were selected and transferred to a continuous flow, aerated hydroponic system and allowed to acclimate for 16–18 h in a background 0.8 mM $CaSO_4$ solution (pH 4.3). The hydroponic system was

[5] Corresponding author: E-mail: jot_smyth@ncsu.edu; Fax, +1-919-515-7422.

maintained at 26°C. Lighting was provided by metal halide and sodium vapor lamps in a day/night photoperiod of 8/16 h, at average photosynthetic photon flux density of 550 μmol $m^{-2}$ $s^{-1}$.

*Solution treatments*

After the seedling acclimation period, treatments were established in the 0.8 mM $CaSO_4$ background solution. Aluminum was added from a 100 mM $AlCl_3$ stock solution in diluted HCl in amounts to achieve specific activities of $Al^{3+}$ in the solutions as predicted by the GEOCHEM-PC software (Parker et al. 1995). Calcium and Mg were added as $CaCl_2$ and $MgCl_2$, respectively. The final composition of the solutions are summarized in Silva et al. (2001c). Root elongation was determined by measuring root length at the beginning and end of the experiments, which lasted 72 h unless stated otherwise. Data on the effects of solution treatments on root elongation are reported in Silva et al. (2001c) for experiments carried out independently under similar conditions as those reported herein.

*Extraction and analysis of root tip organic acids*

Root tip content and exudation of organic acids for both soybean genotypes was measured 72 h after imposing the solution treatments, unless stated otherwise. Seedlings were removed from the hydroponic chambers and root tips (0–5 mm) were excised, immediately weighed and then extracted with 80% ethanol using glass grinders. The extracts were brought to dryness using a vacuum-evaporator, and the residue was dissolved in 0.6 ml of high purity water and analyzed for malate and citrate content by ion chromatography using electrical conductivity detection. The complete chromatographic method and system have been described previously (Silva et al. 2001a).

*Collection and analysis of excreted organic acids*

Organic acids secreted by roots were collected after transferring seedlings from the solution chambers to 100 ml vessels containing solutions similar to those in the chambers. The $SO_4^{2-}$ ion interfered with the separation of malate and citrate during the chromatography analysis and could not be effectively removed from the 0.8 mM $CaSO_4$ background solution. Therefore, the background solution in the exudate collection vessels consisted of 0.8 mM $CaCl_2$. The $AlCl_3$ concentration was properly adjusted as predicted with GEOCHEM-PC to obtain final $Al^{3+}$ activities in the exudate collection solution similar to those in the hydroponic chambers.

Solutions were replaced twice during the first hour after seedlings were transferred to the collection vessels. Thereafter, root exudation to treatment solutions were collected at the end of a 6 h period. A similar procedure was employed in time-course experiments, but solutions were drawn off and replaced at 1, 4, 7, 12 and 24 h after the beginning of treatments. All collected solutions were evaporated on a hot plate using a silica-bath (75°C), and the residue was dissolved in 1.0 ml of water and analyzed for citrate and malate by ion chromatography after passing through On Guard-Ag pre-treatment cartridges (Dionex, Sunnyvale, CA, U.S.A.) to remove the excess of $Cl^-$ ions.

*Al-indicator fluorescence microscopy*

The protocol used for the observation of Al accumulation in root tips was similar to that used by Silva et al. (2000). Briefly, root tips of both soybean genotypes were excised from seedlings after 48 h of exposure to treatment solutions in the hydroponic chambers and washed with 10 mM ice-cold citrate for 30 min to remove loosely bound apoplastic Al. Root tips were embedded in 6% agarose and sectioned using a vibrating microtome. Transverse root tip sections 100 μm thick were obtained from a region 100–300 μm behind the tip. The sections were stained with the fluorescent Al-probe lumogallion (Molecular Probes, Eugene, OR) mounted on glass slides and visualized with a 20×/0.60 numerical aperture objective in a confocal laser scanning microscope (Leica Microsystems, Heidelberg, Germany). A Uniphase argon laser was used for visualization of the Al-lumogallion complex, with emitted fluorescence being collected at wavelengths from 500 nm to 550 nm.

*Al, Ca, and Mg accumulation in root apices*

After seedlings were exposed for 48 h to solutions containing 0 or 2.9 μM $Al^{3+}$ activity and a range of Ca or Mg concentrations, their root tips (0–5 mm) were excised, transferred to centrifuge tubes, weighed, and dried at 65°C. The dry tips were digested overnight in 1 ml of Optima grade $HNO_3$ (Al <10 ng $kg^{-1}$, Fisher Scientific) and microwaved at high power for 30 min under a stream of nitrogen. After dilution with high purity water, Al, Ca and Mg contents were determined by inductively coupled plasma spetrophotometry. Measurements were carried out for three independent replicates. To minimize contamination, only polypropylene or teflon lab ware was used after washing with 20% trace metal-grade $HNO_3$ and rinsing with high purity water.

## Results

*Effect of Al, Mg and soybean genotype on tap root tip and excreted citrate*

In both soybean genotypes, the concentration of citrate in tap root tips was affected by $Al^{3+}$ activity and the presence of Mg in the solution (Fig. 1). In the absence of Mg, root tip concentration of citrate of the PI was at lower levels than that of cv Young in the absence of Al and increased with Al additions up to a 1.5 μM solution $Al^{3+}$ activity. Citrate concentration in cv Young root tips decreased progressively with increasing solution $Al^{3+}$ activity. Both genotypes had similar concentrations of citrate in root tips in treatments of 4.7 μM $Al^{3+}$ activity. In the presence of 50 μM Mg, root tip concentration of citrate of both genotypes increased with $Al^{3+}$ activity in solution (Fig. 1).

Citrate exudation by soybean roots was dependent on solution $Al^{3+}$ activity and Mg concentration in solution, and differed between genotypes (Fig. 2). In the absence of Mg, there was a dose-dependent increase in citrate exudation, which was always greater in the Al-tolerant PI than in the Al-sensitive cv Young. The addition of 50 μM Mg to the rooting medium increased citrate efflux, especially in solutions with 1.5 and 4.7 μM $Al^{3+}$ activity. Although Mg increased the efflux of citrate for both genotypes, values for cv Young were less than for PI but greater than the rates for PI in the absence of Mg.

*Ca and Mg dose effect on tap root tip malate and citrate concentrations*

There were no consistent differences between genotypes for root tip malate concentration following exposure to solutions with variable Ca and Mg supply (Fig. 3). When only 2.9 μM $Al^{3+}$ was present in the basal solution, malate concentrations in root tips of the PI and cv Young were similar. Maximum concentrations of malate in root tips of PI were found in solutions treatments with 0.5 mM Ca or 10 μM Mg. Similar responses were observed in root tip malate concentrations for

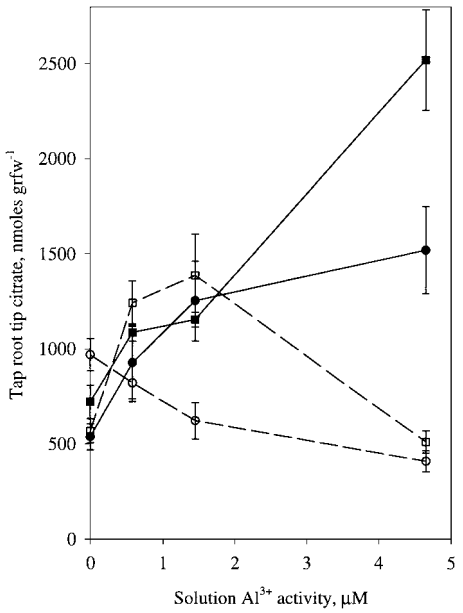


**Fig. 1** Concentration of citrate in tap root tips of soybean genotypes Young (open and filled circles) and PI 416937 (open and filled squares) after 72 h of exposure to an 0.8 mM $CaSO_4$ solution (pH 4.3) containing variable $Al^{3+}$ activities, in the absence (open symbols) or presence (filled symbols) of 50 μM Mg. Vertical bars denote standard errors.

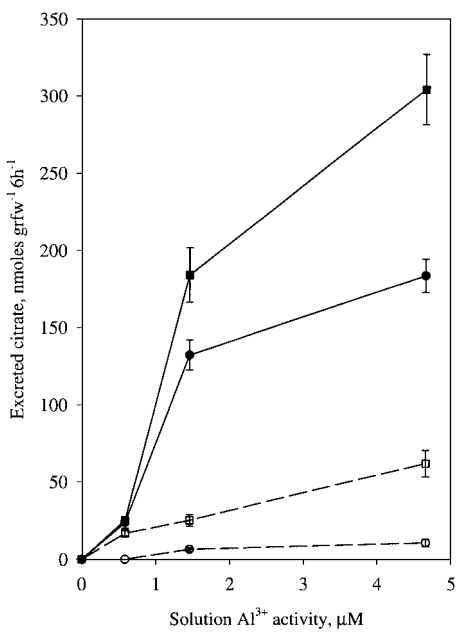


**Fig. 2** Exudation of citrate by roots of soybean genotypes Young (open and filled circles) and PI 416937 (open and filled squares) after 72 h of exposure to an 0.8 mM $CaSO_4$ solution (pH 4.3) containing variable $Al^{3+}$ activities, in the absence (open symbols) or presence (filled symbols) of 50 μM Mg. Vertical bars denote standard errors.

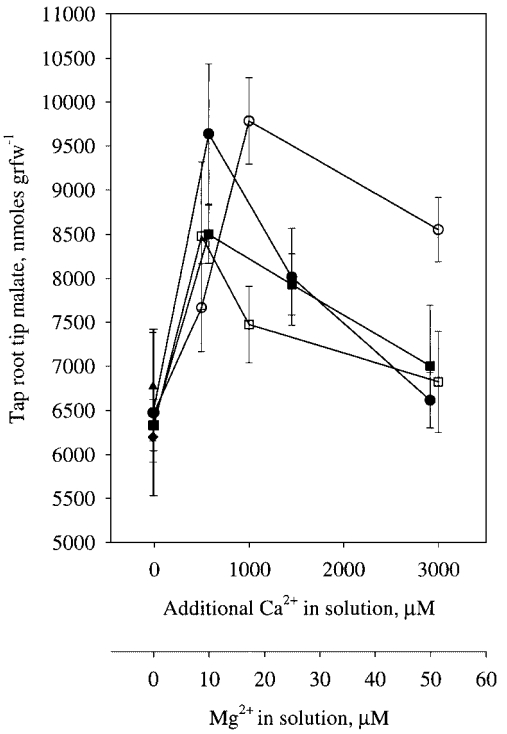


**Fig. 3** Concentration of malate in tap root tips of soybean genotypes Young (open circles, filled circles, filled triangles) and PI 416937 (open squares, filled squares, filled diamonds) after a 72 h exposure to an 0.8 mM $CaSO_4$ solution at pH 4.3, in either the absence of Al (filled triangles, filled diamonds) or presence of 2.9 μM $Al^{3+}$ and supplemented with variable Ca (open circles, open squares) and Mg (filled circles, filled squares) concentrations. Vertical bars denote standard errors.

cv Young. In solutions with high Ca or Mg, malate concentrations in root tips of both genotypes decreased to levels similar to the control treatments with only the background 0.8 mM $CaSO_4$ solution.

Root tip citrate concentrations in both genotypes increased with Ca and Mg additions to treatment solutions (Fig. 4). Maximum citrate concentrations were approached with the addition of 10 μM Mg to solutions but the same level of citrate in the root tip was only achieved with solutions containing 1 mM Ca for PI and 3 mM Ca for cv Young.

*Ca and Mg effect on lateral root tip citrate concentration*

Comparisons of Ca and Mg effects on citrate concentration between lateral and tap root tips were intended to complement our previous observations that lateral roots are more sensitive to Al toxicity than tap roots (Silva et al. 2001a). A 2.9 μM $Al^{3+}$ activity in solution reduced lateral root tip citrate concentration relative to control plants. Additions of 3 mM Ca to solutions with Al had a negligible effect on root tip citrate, whereas 50 μM Mg increased lateral root tip concentration of citrate by almost 4-fold in both genotypes (Fig. 5).

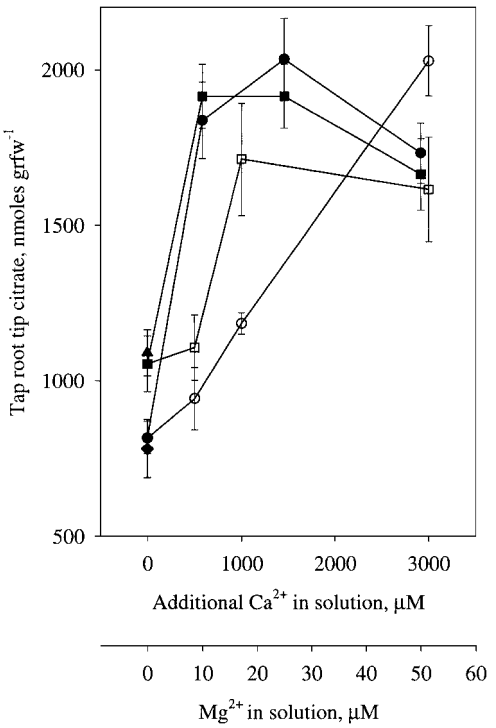


**Fig. 4** Concentration of citrate in tap root tips of soybean genotypes Young (open circles, filled circles, filled triangles) and PI 416937 (open squares, filled squares, filled diamonds) after a 72 h exposure to an 0.8 mM $CaSO_4$ solution at pH 4.3, in either the absence of Al (filled triangles, filled diamonds) or presence of 2.9 µM $Al^{3+}$ and supplemented with variable Ca (open circles, open squares) and Mg (filled circles, filled squares) concentrations. Vertical bars denote standard errors.

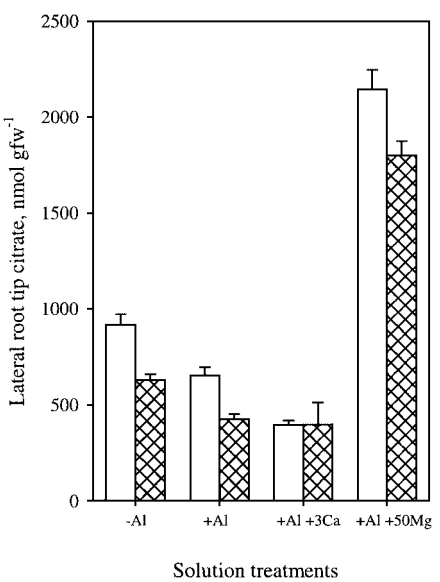


**Fig. 5** Concentration of citrate in lateral root tips of soybean genotypes PI 416937 (open bars) and Young (filled bars) after a 72 h exposure to an 0.8 mM $CaSO_4$ solution at pH 4.3, in either the absence (–Al) or presence of 2.9 µM $Al^{3+}$ (+Al) and supplemented with 3 mM Ca (+Al+3Ca) or 50 µM Mg (+Al+50Mg). Vertical bars denote standard errors.

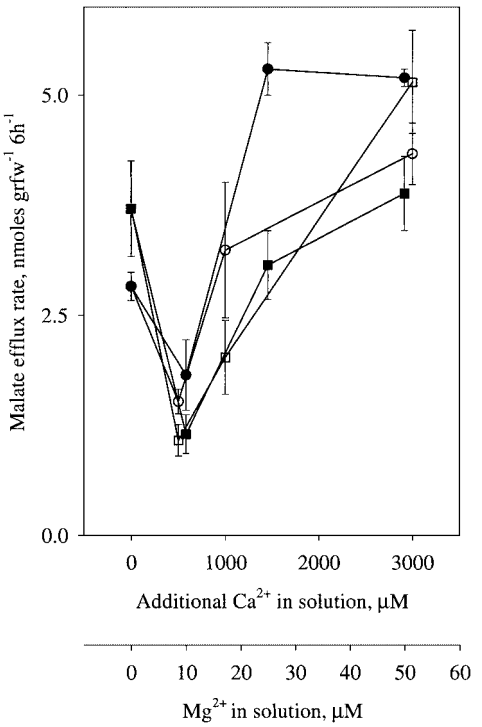


**Fig. 6** Efflux of malate by roots of soybean genotypes Young (open and filled circles) and PI 416937 (open and filled squares) after a 72 h exposure to an 0.8 mM $CaSO_4$ solution at pH 4.3, in the presence of 2.9 µM $Al^{3+}$ and supplemented with variable Ca (open symbols) and Mg (filled symbols) concentrations. Vertical bars denote standard errors.

*Root efflux of citrate and malate in response to Ca and Mg additions*

Malate efflux rates for whole-root systems of both soybean genotypes are presented in Fig. 6. After an initial decrease (with no plausible reason), secretion of malate by roots of both genotypes increased with additions of Mg and supplemental Ca to the background solution with constant $Al^{3+}$ activity. Although malate concentrations were higher than citrate in root tips (Fig. 4), malate efflux rates are several orders of magnitude lower than for citrate (Fig. 7). Another important difference between malate and citrate exudation is that only Mg promoted citrate secretion (Fig. 7). At Mg levels above 20 µM, citrate efflux rates for cv Young were greater than for the PI.

*Time-course of Mg stimulation of citrate efflux*

Citrate efflux by roots of cv Young seedlings was evaluated over 24 h after plants were acclimated in the 0.8 mM $CaSO_4$ basal solution and then exposed to 2.9 µM $Al^{3+}$ both with and without 50 µM Mg in the collection vessels. For a third group of plants 50 µM Mg was added to the 0.8 mM $CaSO_4$ basal solution during the 18 h acclimation period in the hydroponic chambers followed by exposure to 2.9 µM $Al^{3+}$ plus 50 µM Mg in the collection vessels. Citrate efflux rates for cv

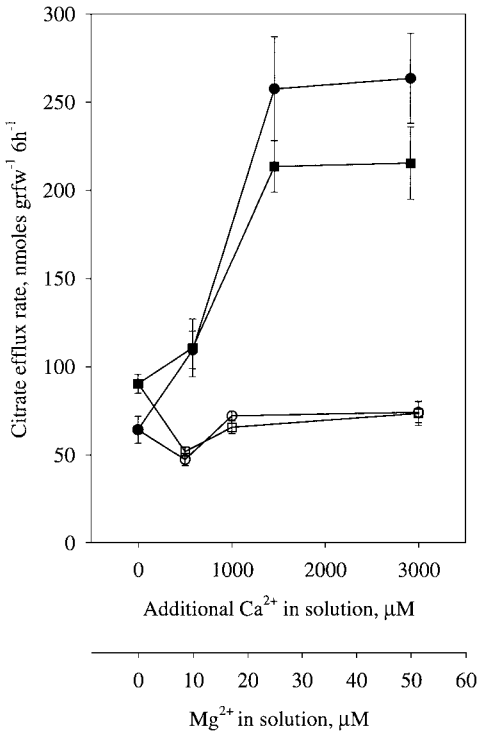


**Fig. 7** Efflux of citrate by roots of soybean genotypes Young (open and filled circles) and PI 416937 (open and filled squares) after a 72 h exposure to a solution (pH 4.3) with 0.8 mM $CaSO_4$, 2.9 μM $Al^{3+}$ and supplemented with variable Ca (open symbols) and Mg (filled symbols) concentrations. Vertical bars denote standard errors.

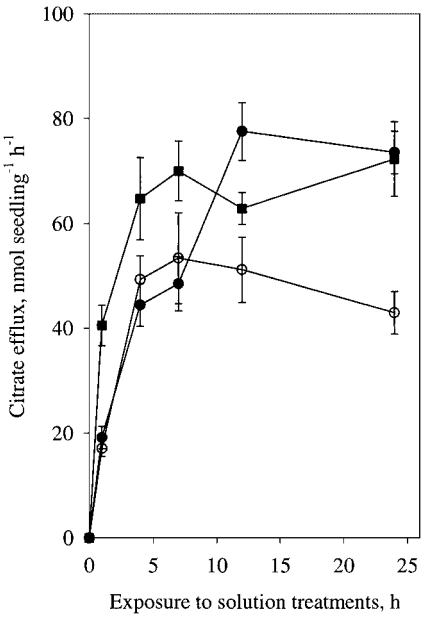


**Fig. 8** Efflux of citrate by roots of soybean cv. Young with time of exposure to solutions (pH 4.3) with 2.9 μM $Al^{3+}$, 0.8 mM $CaSO_4$ and either no Mg (open circles), 50 μM Mg (filled circles) or pre-exposure for 18 h to 50 μM Mg (filled squares). Vertical bars denote standard errors.

Young roots exposed to the three treatment solutions as a function of time are shown in Fig. 8. Among treatments without pre-exposure to Mg, no significant differences in citrate efflux were observed between roots exposed to 2.9 μM $Al^{3+}$ with or without Mg in the collection vessels up to 7 h after imposing the treatments. After this period, however, citrate efflux by plants exposed to 2.9 μM $Al^{3+}$ and no Mg leveled off and even tended to decrease, while an increase in citrate efflux was observed for plants exposed to 2.9 μM $Al^{3+}$ in the presence of 50 μM Mg. Plants that were pre-conditioned for 18 h in the presence of Mg (0 μM $Al^{3+}$) in the hydroponic chamber followed by exposure to solutions containing 2.9 μM $Al^{3+}$ in the presence of 50 μM Mg secreted greater amounts of citrate as early as 1 h after starting collection of exudates. Although the initial citrate efflux rate by seedlings pre-exposed to Mg for 18 h was greater than for other solution treatments, approximately 7 h were required to reach near maximum citrate efflux rates (Fig. 8).

*Solution treatment effects on Al, Ca and Mg accumulation in tap root tips*

Fluorescence images of the Al probe lumogallion indicate that solution treatments affected Al accumulation in root tips of both soybean genotypes (Fig. 9A–I). In root sections from plants exposed to 0 μM $Al^{3+}$ low levels of fluorescence were detected and the image is virtually black (Fig. 9A). A higher fluorescence was observed for roots of both genotypes exposed to 2.9 μM $Al^{3+}$ (Fig. 9F, G). The fluorescence in Al-treated roots decreased with increasing additions of Mg to the solutions, indicating a reduction in Al accumulation in this root region. Low fluorescence, thus low Al, was detectable in the treatments with 50 μM solution Mg. Roots of both genotypes exposed to 3 mM Ca also had reduced levels of Al, with accumulation of Al similar to those of roots exposed to 2.9 μM $Al^{3+}$ and 50 μM Mg (compare Fig. 9D, 9E with 9H, 9I).

Quantitative spectrometric analyses of Al, Ca and Mg in tap root tips were performed to complement the fluorescence data. Levels of Al in root tips of control plants were almost undetectable, whereas the presence of 2.9 μM $Al^{3+}$ activity in solutions significantly increased the Al concentration in root tips of the PI and cv Young (Fig. 10A). With 2.9 μM $Al^{3+}$ activity and no additional cations in solution, root apices of both genotypes were visually swollen and damaged and elongation was severely restricted. Supplementation of the background solution with 3 mM Ca or 50 μM Mg minimized Al accumulation in root tips of both genotypes, although this concentration of Ca was slightly more efficient than 50 μM Mg (Fig. 10A). Exposure of roots to solutions with 2.9 μM $Al^{3+}$ activity had no negative effect on Ca concentration in root tips of cv Young and the PI (Fig. 10B) but Mg concentration in root tips of both genotypes was reduced by more than 50% when compared to plants in the control solutions (Fig. 10C).

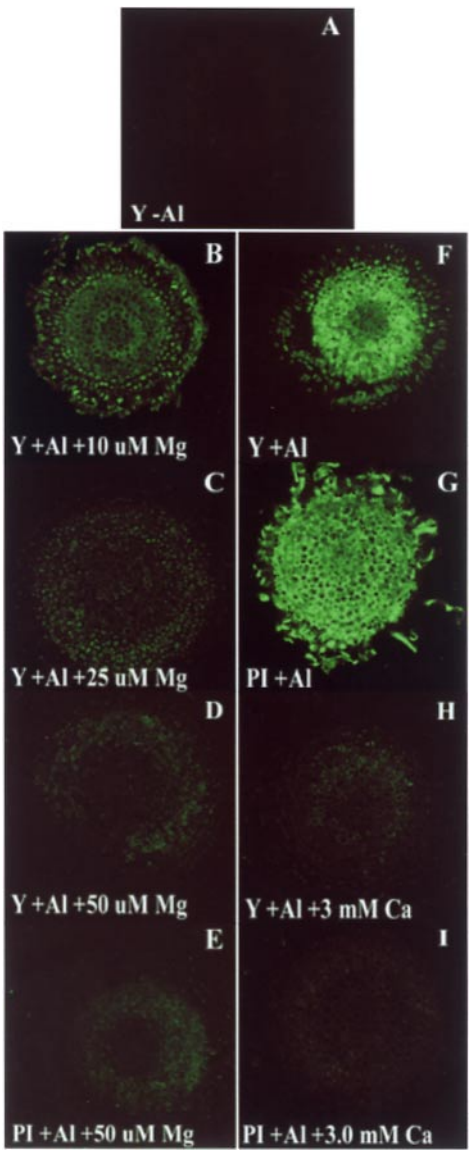


**Fig. 9** Aluminum-lumogallion fluorescence demonstrating Al accumulation in tap root tip cross sections of soybean genotypes PI 416937 (PI) and Young (Y) after 72 h of exposure to an 0.8 mM $CaSO_4$ solution (pH 4.3), in the absence (A) or presence of 2.9 μM $Al^{3+}$ and supplemented with variable Ca (F–I) and Mg (B–E) concentrations.

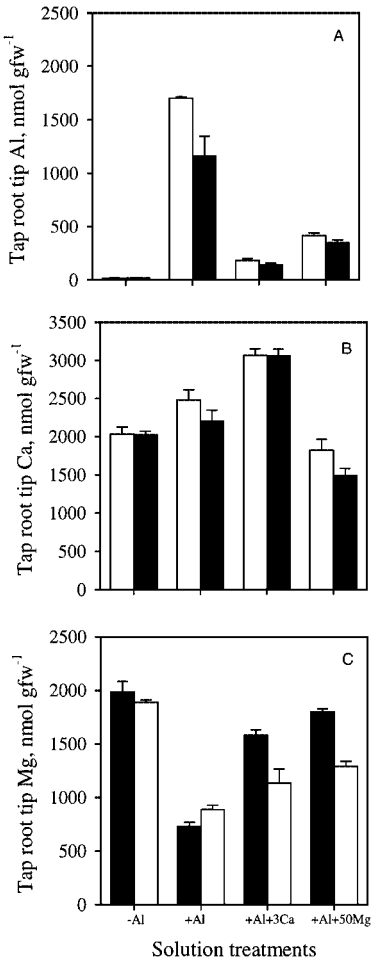


**Fig. 10** Aluminum (A), Ca (B) and Mg (C) concentrations in tap root tips of soybean genotypes PI 416937 (PI) (open bars) and Young (filled bars) after 72 h of exposure to an 0.8 mM $CaSO_4$ solution (pH 4.3), in either the absence of Al (–Al) or presence of 2.9 μM $Al^{3+}$ (+Al) and supplemented with 3 mM Ca (+Al+3Ca) or 50 μM Mg (+Al+50Mg). Vertical bars denote standard errors.

## Discussion

For soybean seedlings growing in the 0.8 mM $CaSO_4$ background solution with constant $Al^{3+}$ activities, the addition of 50 μM Mg significantly increased root tip concentration (Fig. 1, 4) and root secretion of citrate (Fig. 2, 3), and this paralleled the effects observed on tap root elongation (Silva et al. 2001c). The Mg effect appears to be somewhat organic acid-specific since increasing Mg additions to solutions containing inhibitory $Al^{3+}$ activities increased the root tip concentration and efflux of citrate to the solution but resulted in relatively smaller changes for malate exudation and concentration in the root tip. Changes in malate, however, were not related to the effects on root elongation. This behavior is in agreement with previous observations that citrate seems to be the organic acid associated with Al tolerance in soybean (Yang et al. 2000, Silva et al. 2001a). Supplementation of Ca to the basal solution also increased tap root tip content of citrate in both genotypes, but no substantial effect was observed on citrate secretion by roots.

Several hypothesis have been proposed to explain the mechanistic basis of cation amelioration of Al toxicity. There is evidence that Ca and Mg, most frequently in the milimolar range, alleviate Al toxicity by electrostatic effects, wherein increases in the root electrical potential reduce $Al^{3+}$ activity at the plasma membrane surface (Kinraide et al. 1992, Kinraide

1998) and Al uptake (Vitorello and Haug 1996, Ryan et al. 1997a). Although Ca was an effective promoter of tap root elongation of both genotypes at high concentrations (mM range), the most striking finding of our study is that micromolar concentrations of Mg enhanced the citrate concentration at the root tip and stimulated citrate secretion by roots exposed to toxic levels of Al. An increased Al tolerance in the presence of low Mg levels could, thus, result from detoxification of Al in the rhizosphere through formation of non-toxic Al-citrate complexes. With higher citrate concentrations in the root tip, Al that entered the cell would be less available to exert toxic effects because of the higher ligand availability.

The ameliorative properties of Ca and Mg in terms of reducing Al uptake are supported by the Al-lumogallion fluorescence images showing the reduction of Al accumulation in the root tip. The addition of 50 μM Mg was as effective as 3 mM Ca in reducing Al accumulation in root tips, although Al is still visible in the cell wall and cytosol of cells. Since root elongation in the presence of otherwise inhibitory $Al^{3+}$ activities in Mg-treated solutions was similar to control levels, one may speculate that the Al was bound in non-toxic forms perhaps as an Al-organic acid complex (Ma 2000).

Greater citrate efflux by roots exposed to Al in the presence of Mg in solution was detected as early as 12 h after beginning the treatments. Moreover, pre-treatment of soybean seedlings with Mg during the acclimation period and exposure to Al in the presence of Mg during the exudate collection period allowed plants to secrete citrate at higher rates during the first hour (Fig. 9). These results indicate that Mg effects are relatively fast. Nevertheless, the 7 h delay required to reach maximum citrate efflux rates indicates that root cells need enough time to take up Mg which can then stimulate citrate production and exudation. Alternatively, Mg could be taken up rapidly, but there is a time delay for activation of enzymes, protein synthesis, or even up-regulation of genes involved in the citrate synthesis.

Our results suggest the possibility that pre-treating plants with Mg in the absence of Al 'primes' the mechanism of citrate production for subsequent exposure of roots to Al. Citrate synthase does not seem to require Mg for activation, but the metal stimulates four other key enzymes in the tricarboxylic acid cycle (Heaton 1990). It is not known, however, if stimulation of enzymes actually is required for increased organic acid release. In short-term (1 h) experiments, malate secretion by root tips of an Al-tolerant wheat genotype was not related to the activity of enzymes involved in malate synthesis (Ryan et al. 1995), although de novo malate synthesis occurred in both short (Delhaize et al. 1993) and longer term studies (Basu et al. 1994). Thus, if the presence of Mg is able to stimulate the whole tricarboxylic acid cycle, which is consistent with the increase in root tip malate and citrate concentrations observed in the present study, one puzzling question that remains is why malate secretion is not stimulated to the same extent as citrate (Fig. 6). Studies with other plant species indicate that Al affects secretion of distinct organic acids by roots with different magnitudes (Delhaize et al. 1993, Pellet et al. 1995, Larsen et al. 1998, Zheng et al. 1998). The possibility exists that exposing soybean roots to Al selectively stimulates the efflux of citrate, perhaps by affecting the gating properties of citrate-carrying channels or, in a longer term prospective, the synthesis of the plasma membrane channel protein involved in citrate release. Magnesium could both stimulate citrate production as proposed above and stimulate citrate release by affecting the regulation of the putative citrate channel. Several investigations suggested that G-proteins are a critical target for Al toxicity (Macdonald and Martin 1988, Haug et al. 1994), and there is experimental evidence that the positive effect of Mg against Al toxicity could involve alleviation of Al-induced G-protein inhibition (Landino and Macdonald 1997). In both mammalian and plant systems, there is evidence that Mg modulates the opening of ion channels (Yamaoka and Seyama 1996, Brüggemann et al. 1999) and that such regulation seems to be dependent on normal G-protein activity (Ruhfus et al. 1996, Hebe et al. 1999, Leaney et al. 2000). Although Al could activate citrate release by direct interaction with a putative citrate channel in the external face of the plasma membrane, the Mg modulation of the putative citrate channel would occur from the cytoplasmic side, perhaps involving Mg-dependent G-proteins. Future patch-clamp studies with Al-activated anion channels (Ryan et al. 1997b, Piñeros and Kochian 1999), either in the absence or presence of Mg and G-protein activators and inhibitors, should further our understanding of the Mg-stimulated citrate secretion reported in the present study.

Under conditions of Al stress, one of the most common symptoms of toxicity is Ca deficiency (Foy 1992, Rengel 1992). This has led several researchers to propose blockage of Ca uptake as the primary lesion involved in Al toxicity to plants (Rengel 1992, Rengel et al. 1995). In our experiments, exposure of soybean roots to 2.9 μM $Al^{3+}$ in a 0.8 mM $CaSO_4$ basal solution failed to reduce Ca concentration at the root tip (Fig. 10B) while drastically inhibiting root elongation (Silva et al. 2001c). Accordingly, low Al levels (2 μM) had no effect on Ca influx in root hair tips of *Limnobium stoloniferum* although growth had ceased (Jones et al. 1995). In a study with the giant alga *Chara corallina*, Ca influx was only slightly affected by Al concentrations that significantly reduced growth (Reid et al. 1995). Other studies with crop species also have shown that Al in solution hardly affected whole-root (Tan et al. 1992) or root tip (Ryan et al. 1997a) Ca concentrations. Nevertheless, the possibility remains that Al could affect root growth through disturbance of Ca homeostasis of root tip cells (Zhang and Rengel 1999).

In contrast with Ca, exposure to Al in the absence of Mg additions did decrease the concentration of Mg at the tap root apices of both genotypes (Fig. 10C). Since Mg was not included in the 0.8 mM $CaSO_4$ solution treatment, Al may have interfered with Mg translocation to the root tip from the cotyledons and mature root regions, or Mg efflux. Supplement-

ing the basal solution with Mg not only raised root tip Mg concentration to the control levels (Fig. 10C), but also enhanced root growth (Silva et al. 2001c). It thus appears that Al-toxicity could be due to both inhibition of Mg uptake (Rengel and Robinson 1989, Fowler et al. 1999) and a reduction in Mg translocation for rapidly dividing and extending cells at the root apex.

Our hypothesis that Mg promotes both external and internal detoxification by formation of Al-citrate complexes finds support in previous studies with the ameliorative ion Si. Silicon alleviates Al toxicity by preventing Al accumulation in roots, and this effect does not appear to depend on a reduction of the Al level by precipitation of insoluble Al-Si compounds in the external solution (Corrales et al. 1997, Hara et al. 1999, Cocker et al. 1998a). Additionally, Si treatment in the presence of Al significantly increased malate concentration in teosinte roots (Barceló et al. 1993). Hypotheses were recently proposed integrating organic acid secretion by roots and formation of non-toxic Al complexes in root tip apoplast of Si-treated plants (Corrales et al. 1997, Cocker et al. 1998b). Increased production and release of citrate by soybean roots in response to Mg supply could play a similar role in protecting against Al damage by minimizing both Al uptake and enhancing internal detoxification of Al that had entered the root tip cells.

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